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The Influence of HLA Class I Alleles and Heterozygosity on the Outcome of Human T Cell Lymphotropic Virus Type I Infection¹

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The inflammatory disease human T cell lymphotropic virus type I (HTLV-I)-associated myelopathy (HAM/TSP) occurs in only 1–2% of HTLV-I-infected individuals and is associated with a high provirus load of HTLV-I. We hypothesize that a person's risk of developing HAM/TSP depends upon the efficiency of their immune response to the virus, which differs between individuals because of polymorphism in genes that influence this response. Previously we showed that the possession of *HLA-A*02* was associated with a lower risk of HAM/TSP, and with a lower provirus load in healthy carriers of HTLV-I. However, *HLA-A*02* did not account for all the observed difference in the risk of HAM/TSP. Here we present evidence, in the same study population in Japan, that *HLA-Cw*08* was also associated with disease protection (probability value, two-tailed test = 0.002) and with a lower proviral load in healthy carriers. Possession of the *A*02* and/or *Cw*08* genes prevented 36% of potential HAM/TSP cases. In contrast, *HLA-B*5401* was associated with a higher susceptibility to HAM/TSP (probability value, two-tailed test = 0.0003) and with a higher provirus load in HAM/TSP patients. At a given provirus load, *B*5401* appeared to increase the risk of disease. The fraction of HAM/TSP cases attributable to *B*5401* was 17%. Furthermore, individuals who were heterozygous at all three HLA class I loci have a lower HTLV-I provirus load than those who were homozygous at one or more loci. These results are consistent with the proposal that a strong class I-restricted CTL response to HTLV-I reduces the proviral load and hence the risk of disease. *The Journal of Immunology*, 2000, 165: 7278–7284.

The inflammatory disease, human T cell lymphotropic virus type I (HTLV-I)⁴-associated myelopathy/tropical spastic paraparesis (HAM/TSP), is caused by infection with HTLV-I. Of the 10–20 million people infected with the virus, ~95% remain healthy carriers (HCs), 2–3% develop HAM/TSP, and another 1–2% develop an aggressive adult T cell leukemia. The factors that cause these different manifestations of HTLV-I infection are not yet fully understood. The immune response to the virus is characterized by a chronically activated CTL response in most infected people, and a strong Ab response. The CTL response

is directed mainly at the HTLV-I protein Tax, a powerful transactivator of viral transcription (1–3). Tax also transactivates many host cell genes, for example, IL-2 and IL-2 receptor (4, 5). A major risk factor for HAM/TSP is the proviral load; the median proviral load is 16 times higher in HAM/TSP patients than in HCAs (6). A high HTLV-I proviral load is also associated with an increased risk of progression to disease (6, 7). However, we have suggested that CTLs might be able to limit replication of the virus and so determine the provirus load and the risk of proinflammatory disease (8, 9).

It is possible that HTLV-I-specific CTLs exert both protective and inflammatory effects. There is a precedent for this in influenza virus infection in the mouse, where the anti-influenza CTL protected against disease after a low dose of virus, but exacerbated viral pathology at a high dose (10). There is also evidence that HTLV-I-specific CTL could contribute to the inflammation seen in HAM/TSP. Inflammatory cytokines and chemokines, including IFN- γ and TNF, are produced by the frequent HTLV-I-specific CD8⁺ T cells in peripheral blood and in spinal cord lesions (11–14). Such CD8⁺ T cells could cause bystander damage to cells in the CNS (11, 15).

However, frequent and chronically activated HTLV-I-specific CTLs have been found in HCAs as well as in HAM/TSP patients (2, 16–18). We suggested that CTLs were protective in HCAs because the *tax* gene, which encodes the dominant CTL target Ag (1–3), was subject to positive selection in these individuals (19). Recently we have shown that freshly isolated, naturally infected CD4⁺ cells capable of expressing Tax were rapidly killed by virus-specific CTLs in vitro using a perforin-dependent mechanism. This is consistent with the view that the CTLs efficiently destroy HTLV-I-infected cells in vivo and so protect against inflammatory diseases such as HAM/TSP (20).

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⁴ Abbreviations used in this paper: HTLV-I, human T cell lymphotropic virus type I; HAM/TSP, HTLV-I associated myelopathy/tropical spastic paraparesis; HCAs, healthy carriers; OR, odds ratio; 2p, probability value, two-tailed test; CI, confidence interval; Fp, prevented fraction of disease; SSP, sequence-specific primer; MICA, MHC class I chain-related gene A.

Host genetic factors are major determinants of susceptibility to infectious disease (21); the HLA complex plays a particularly important role. We have taken an interest in HLA class I associations with HTLV-I-associated diseases because of the presence of a powerful CTL response in HTLV-I infection. Previous HLA studies in HTLV-I infection have found an association between *DRB1*0101* and other genes on the *HLA-B*0702-Cw*0702-DRB1*0101-DQB1*0501* haplotype and susceptibility to HAM/TSP in the Japanese population (22–24). In a recent case-control study, we showed that the MHC class I gene *HLA-A*02* conferred protection from HAM/TSP; possession of *HLA-A*02* halved the odds of HAM/TSP for a person infected with HTLV-I, and the *A*02* allele prevented ~28% of potential cases in the study population (18). These observations suggested that *A*02*-restricted CTL are particularly efficient at recognizing Tax, and we found that *A*02*-positive HCs had a provirus load one-third that of *A*02*-negative carriers (18). Furthermore, the increased susceptibility to HAM/TSP associated with the *DRB1*0101* haplotype was evident only in *A*02*-negative subjects. Therefore, we conclude that host genetic factors do indeed influence both a person's provirus load and the risk of HAM/TSP.

The first aim of this study was to examine the frequency of class I alleles in the study population that showed a suggestive difference between patients and controls ($0.05 < 2p \leq 0.10$; where $2p$ denotes probability value in a two-tailed test) in our initial study (*Cw*08*, *A*11*). Because the class I-restricted T cell response appears to play an important part in deciding the outcome of HTLV-I infection, we also wished to test the hypothesis that heterozygosity at HLA class I loci is protective in HTLV-I infection. An individual who has two different alleles at each HLA locus can present a wider repertoire of antigenic peptides to the CTL than a homozygote. This could result in a more efficient CTL response to HTLV-I, a lower proviral load, and a lower risk of disease. Heterozygote advantage has recently been demonstrated in HIV (for class I loci) (25, 26) and hepatitis B infection (for class II loci) (27).

Our results show that *HLA-Cw*08* protects against HAM/TSP and is associated with an almost 4-fold reduction in provirus load. The *Cw*08* effect was independent of and additive to the *A*02* effect shown previously. *HLA-B*5401* was associated with an increase in the risk of disease and a higher provirus load in HAM/TSP patients. The protective effect of *A*02* seen previously had no effect on the susceptibility to HAM/TSP associated with *B*5401*. The results also demonstrate a significant effect of HLA class I heterozygosity; HAM/TSP patients heterozygous at all three class I loci had a lower proviral load than individuals homozygous at one or more class I loci.

Materials and Methods

Study population

Two hundred thirty-three cases of HAM/TSP were compared with 202 randomly selected HTLV-I-seropositive asymptomatic blood donors (HCs) from the Kagoshima Red Cross Blood Transfusion Service. All cases and controls were of Japanese ethnic origin and resided in Kagoshima Prefecture, Japan. The diagnosis of HAM/TSP was made according to World Health Organization diagnostic criteria (28).

HLA class I typing

A staged study was performed. In stage 1, 100 cases of HAM/TSP and 100 HCs were studied. In the first 50 cases and 56 controls, PCR-sequence-specific primer (PCR-SSP) reactions were performed to detect all known HLA-A, -B, and -C specificities in an allele- or group-specific manner (96 reactions) (29). The remaining 50 cases and 44 controls were typed with a restricted set of 48 PCR-SSP reactions designed to detect all the HLA-A, -B, and -C specificities that 1) occurred at a gene frequency of $\geq 5\%$, or 2) were associated with an odds ratio (OR) of HAM/TSP of ≤ 0.5 or ≥ 2.0 in the first 50 cases and 56 controls. The results of this initial study, which

showed that *HLA-A*02* is associated with disease protection, and the haplotype *B*0702-Cw*0702-DRB1*0101-DQB1*0501* is associated with susceptibility, have already been published (18).

Stage 2 of the study was designed to test the hypotheses that 1) further class I alleles and 2) HLA class I heterozygosity are associated with protection against HAM/TSP or a reduction in provirus load. We chose to restrict the analysis of the further class I typing to those alleles that showed a suggestive difference in frequency ($0.05 < 2p \leq 0.10$) in stage 1 and those in linkage disequilibrium with alleles associated with disease protection/susceptibility.

HLA heterozygosity study

To determine the heterozygosity in the HLA loci, we conducted a complete HLA class I typing on each subject. Initially, a reduced number of PCR-SSP reactions were performed, as above, to detect all the common alleles. Then, at any class I locus that appeared to be homozygous from the stage 1 or 2 study, the class I HLA typing was completed at each locus to detect the rarer alleles. For the purposes of the heterozygosity study, each locus was studied to the type level only (e.g., *HLA-A*02* rather than *HLA-A*0201*). We compared the HAM/TSP risk and proviral load between individuals who were heterozygous at all three class I loci and those who were homozygous at one or more loci. The power of statistical tests of heterozygosity at individual class I loci was limited by the small number of subjects involved.

Class I subtyping

Following an initial analysis of our results, PCR-SSP reactions were designed to differentiate *Cw*0801* from *Cw*0803/0806* (30), *B*4006* from *B*4002*, and *B*5401* from *B*5507*. The design of SSPs was based on published gene sequences (31) updated from HLA informatics pages available on the internet (<http://www.anthonynolan.com/HIG/index.html>). PCR methods were as previously described (29).

Class II typing

DRB1 and *DQB1* typing was performed as previously described (18).

Detection of single nucleotide polymorphisms in the TNF- α promoter region

A 314-bp fragment of the TNF- α 5' flanking region (incorporating the T-1031C, C-863A, and C-857T single nucleotide polymorphism (SNP) sites (32), was amplified by primary PCR (primers 5' *agggatatgtgatggactac*; 5' *tattccataactggaggtcc*, designed in house) (GenBank accession number M16441) and sequenced using dRhodamine terminator chemistry (Perkin-Elmer, Norwalk, CT) on an automated DNA sequencer (ABI 377; Perkin-Elmer). A total of 209 HAM/TSP patients and 195 HCs were screened.

Proviral load measurement

The HTLV-I provirus load in PBMC was measured at one time point in all patients and HCs, as described elsewhere (6, 18). A quantitative PCR was performed using an ABI 7700 sequence detector (Perkin-Elmer). The lower limit of detection was one copy of HTLV-I (tax) per 10^4 PBMC.

Statistical analysis

The χ^2 test, Mann-Whitney *U* test, and the OR (GraphPad, San Diego, CA) were used for statistical analysis. Where the number of observations was < 20 in any category, Fisher's Exact test was used for a 2×2 table. The Bonferroni method (33) was used to correct for multiple comparisons. The prevented fraction of disease (Fp), i.e., the fraction of potential cases of disease in the study population that is prevented by a specified factor, was calculated as previously described (18). The population attributable risk, i.e., the fraction of observed cases of disease that is attributable to a specified factor, was calculated according to Schlesselman (34). To calculate the risk of HAM/TSP at a given proviral load, Bayes' theorem of conditional probabilities was used, as detailed in Bangham et al. (8). For clarity, probability levels are cited as follows: $2p$ denotes the results of two-tailed tests, and p denotes a one-tailed test (e.g., $2p = 0.002$).

Results

The age and sex distributions of the patient and control groups were as previously described (18). Although there was an excess of females in the HAM/TSP group and an absence of subjects under 16 or over 65 from the control (blood donation) group, the frequency of occurrence of individual HLA alleles was unaffected by age and sex (data not shown).

Table I. *HLA-Cw*08 reduced the odds of HAM/TSP^a*

	HAM/TSP <i>n</i> (%)		HCs <i>n</i> (%)		<i>2p</i>	OR [†] (95% CI)
	Cw*08 ⁺	Cw*08 ⁻	Cw*08 ⁺	Cw*08 ⁻		
Stage 1	8 (8)	92 (92)	17 (17)	83 (83)	0.087 [#]	0.42 (0.17–1.04)
Stage 2	16 (12)	117 (88)	26 (25)	76 (75)	0.013 [#]	0.40 (0.20–0.79)
All subjects	24 (10)	209 (90)	43 (21)	159 (79)	0.002 [*]	0.42 (0.25–0.73)

^a Cw*08⁺ and Cw*08⁻ denote the presence or absence of the Cw*08 gene in the subjects studied. In total, 233 HAM/TSP patients and 202 HCs were studied. Stage 1 and Stage 2 denote two independent, consecutive case-control studies, and do not refer to clinical stage. *n*, Number of subjects; #, Fisher's Exact Test (two-tailed); *, χ^2 with Yates correction (two-tailed); †, using the approximation of Woolf.

*HLA-Cw*08 was associated with a lower risk of HAM/TSP and a lower proviral load*

Alleles selected from stage 1 for stage 2 of the study were Cw*08 ($2p = 0.087$, OR = 0.42, 95% confidence interval (CI) = 0.17–1.04) (Table I) and A*11 ($2p = 0.09$, OR = 2.07, 95% CI = 0.96–4.45). It was necessary to confirm these associations not only because of the large number of alleles tested for, but because they were not significant at $p < 0.05$. We proceeded to test these associations in an independent sample. The excess of A*11-positive subjects seen in the HAM/TSP subjects in stage 1 was not maintained in stage 2 or in the cohort as a whole (45 of 233 HAM/TSP A*11⁺; 31 of 202 HCs A*11⁺, $2p = 0.33$). However, in the second stage of the study, and overall, the genotype frequency of HLA-Cw*08 was significantly lower among the cases of HAM/TSP compared with the controls (Table I). The possession of HLA-Cw*08 was associated with a >2-fold reduction in the odds of HAM/TSP ($2p = 0.002$, OR = 0.42, 95% CI = 0.25–0.73). Given this OR and the observed frequency of Cw*08 in Kagoshima, we can estimate the proportion of potential cases of HAM/TSP that are prevented by the presence of Cw*08 (the Fp; Ref. 18). Here, Fp = 12.6% ($\pm 3.7\%$ SD). We then tested the hypothesis that if a gene is associated with protection from disease, it is also associated with a reduction in provirus load in HCs of the virus, as the risk of developing disease is dependent on the provirus load (6, 7). There was a significant reduction in median provirus load of almost 4-fold associated with Cw*08 in the HCs ($2p = 0.046$, Mann-Whitney *U* statistic) (Table II).

Cw*08 subtyping was conducted to see whether there was an association between protection against HAM/TSP and a particular subtype. Subtypes detected were Cw*0801, Cw*0802, and Cw*0803/06. The genotype frequencies were as follows; Cw*0801: HAM/TSP 9.4%, HCs 18.3%; Cw*0802: HAM/TSP 0%, HCs 0.5%; Cw*0803/06: HAM/TSP 0.9%, HCs 2.5%. The predominant allele, HLA-Cw*0801, was significantly associated with disease protection in comparison with Cw*08-negative subjects ($2p = 0.008$, OR = 0.45, 95% CI = 0.26–0.80) and an almost 5-fold reduction in provirus load in the HCs ($2p = 0.028$, Mann-Whitney *U* statistic) (Table II).

*The protective effect of Cw*08 was independent of and additive to the effect of A*02*

Three lines of evidence suggest that the effect of HLA-Cw*08 was independent of and additive to those of A*02: 1) A*02 and Cw*08 were not in significant linkage disequilibrium in the HCs (data not shown); 2) Cw*08 reduced the odds of disease in A*02-negative subjects ($2p = 0.013$) (Table III) and A*02 reduced the odds of disease in Cw*08-negative subjects ($2p < 0.0001$, OR = 0.41, 95% CI = 0.26–0.63) (data not shown); and 3) Cw*08 was associated with a >3-fold reduction in median provirus load in both A*02-positive and -negative HCs using the Mann-Whitney *U* statistic (median proviral

load in A*02-positive subjects: Cw*08⁺ 6.0 and Cw*08⁻ 25.7 copies/10⁴ PBMC, $p = \text{NS}$; A*02-negative subjects: Cw*08⁺ 15.8 and Cw*08⁻ 53.7 copies/10⁴ PBMC, $p = \text{NS}$).

*Possession of Cw*08 was sufficient to explain the association of other alleles in linkage disequilibrium with Cw*08*

HLA-Cw*08 has been reported to be in linkage disequilibrium in the Japanese population with A*02, A*2402, A*2601, and B*48, and with the haplotype B*4006-DRB1*0901-DQB1*0303-DPB1*0201 (35, 36). In this study we found Cw*08 to be in significant linkage disequilibrium in both the HAM/TSP and HC populations with A*26, B*48, B*4006, DRB1*0901, and DQB1*0303 (data not shown). HLA-B*48 was associated with a reduction in the odds of HAM/TSP ($2p = 0.037$, corrected p -NS, OR = 0.39). However, there was no effect of B*48 on provirus load in HCs. Cw*08 was found more frequently than B*48, and B*48 occurred in only three HAM/TSP patients and no HCs in the absence of Cw*08. We conclude that the association of B*48 with disease protection is due to its strong linkage disequilibrium with Cw*08. Other alleles found to be in linkage disequilibrium in this population had no effect on the odds of disease either by themselves or in association with Cw*08.

Heterozygosity at HLA class I loci was associated with a lower proviral load of HTLV-I

We hypothesized that individuals who were heterozygous at all three HLA class I loci would have a lower provirus load than individuals who were homozygous at one or more loci, and this was indeed the case in the HAM/TSP patients ($2p = 0.017$, Mann-Whitney *U* statistic) (Table IV). In the HCs the proviral load was significantly lower in association with full heterozygosity only at a one-tailed level ($p = 0.039$, Mann-Whitney *U* statistic) (Table IV). In addition, homozygosity at the HLA-C locus was associated with a higher provirus load in the HAM/TSP patients (proviral load: heterozygous at HLA-C 530.9 copies/10⁴ PBMC, homozygous at HLA-C 861.0 copies/10⁴ PBMC, $2p = 0.018$, Mann-Whitney *U* statistic). The effect of class I heterozygosity on proviral

Table II. *HLA-Cw*08 and subtype Cw*0801 were associated with a lower median provirus load in HCs^a*

	HAM/TSP		HCs	
	Proviral load [‡] (<i>n</i>)	$2p^{\S}$	Proviral load [‡] (<i>n</i>)	$2p^{\S}$
Cw*08 ⁺	467.7 (23)	0.27	12.0 (43)	0.046
Cw*08 ⁻	575.4 (201)		45.7 (159)	
Cw*0801 ⁺	467.7 (21)	0.36	7.94 (37)	0.028
Cw*08 ⁻	575.4 (201)		45.7 (159)	

^a *n*, Number of subjects; ‡, median proviral copy number per 10⁴ PBMC; §, p level (two-tailed, uncorrected) (Mann-Whitney *U* test).

Table III. *HLA-Cw*08 reduced the odds of HAM/TSP in A*02-negative subjects^a*

	HAM/TSP (n)		HCs (n)		2p [#]	OR [†] (95% CI)
	Cw*08 ⁺	Cw*08 ⁻	Cw*08 ⁺	Cw*08 ⁻		
A*02 ⁻	11	153	17	84	0.013	0.36 (0.16–0.79)
A*02 ⁺	13	56	26	75	0.387	0.67 (0.32–1.42)

^a n, Number of subjects; #, using Fisher's Exact Test (two-tailed); †, using the approximation of Woolf.

load was too small to have a significant effect on odds of HAM/TSP ($p = \text{NS}$, OR = 0.98).

*HLA-B*54 was associated with increased susceptibility to HAM/TSP and a higher proviral load of HTLV-I*

In a parallel study of non-HLA candidate genes in the development of HAM/TSP we identified a suggestive increase in the frequency of HAM/TSP patients carrying the *TNF* –857T allele ($p = 0.08$, OR = 1.46, 95% CI 0.98–2.19). The results from the analysis of the other *TNF* promoter polymorphisms will be presented separately (C. Bangham, A. Vine, A. Witkover, Y. Furukawa, A. Lloyd, K. Jeffery, A. Siddiqui, K. Usuku, and M. Osame. Manuscript in preparation). *TNF* –857T is in linkage disequilibrium with *B*5401* and *DRB1*0405* in the Japanese (32) (extended haplotype *A*2402-Cw*0102-B*5401-DRB1*0405-DQB1*0401*; Ref. 36). Therefore, we examined the alleles in linkage disequilibrium with *TNF* –857T. *B*5401* was significantly associated with disease susceptibility ($2p = 0.0003$ uncorrected, OR = 2.51) (Table V). A correction factor of 48 was applied to the p value for *B*5401* to reflect the number of alleles tested for, to avoid artifacts due to multiple comparisons (33); the p value remains significant at $2p = 0.014$. None of the other alleles in the extended haplotype described above had a significant association with disease (for *A*24*: $2p = 0.145$; *Cw*01*: $2p = 0.150$; *DRB1*0405*: $2p = 0.289$; *DQB1*0401*: $2p = 0.123$). The population-attributable risk of *B*5401*, i.e., the fraction of cases of HAM/TSP that were attributable to *B*5401*, was 16.8% (95% CI = 8.3–24.4%). Table V also shows that *B*5401* was associated with disease susceptibility in both *HLA-A*02*-positive and *HLA-A*02*-negative populations. Conversely, *A*02* was not associated with disease protection in the *B*5401*-positive population, but remained highly significantly associated in the *B*5401*-negative population ($2p = 0.0001$).

As well as being associated with disease susceptibility, *B*5401* was also associated with a significantly higher provirus load in all HAM/TSP patients, and also in the *A*02*-negative HAM/TSP patients, although these increases were no longer significant after correction for multiple comparisons (Table VI). In our previous study, a significantly lower provirus load was seen in association with *A*02* in the HCs. In this study the possession of *A*02* was associated with a lower proviral load (>4-fold) in the *B*5401*-negative HCs, but in the presence of *B*5401* no change in the *A*02*-associated provirus load was seen (Table VI).

Possession of *B*5401* was associated with increased susceptibility to HAM/TSP in *Cw*08*-negative subjects ($2p = 0.0025$, OR = 2.27, 95% CI = 1.34–3.84) but not in *Cw*08*-positive subjects. Conversely, possession of *Cw*08* was associated with protection against HAM/TSP in *B*5401*-negative subjects ($2p = 0.0062$, OR = 0.43, 95% CI = 0.24–0.78). *Cw*08* did not appear to protect in *B*5401*-positive subjects; however, the power of this test was limited by a small number of *B*5401*-positive, *Cw*08*-positive subjects.

*B*5401 was associated with a higher risk of HAM/TSP at a given HTLV-I load*

The data on provirus load can be used to calculate the risk (prevalence) of HAM/TSP at a given provirus load (Fig. 1). As can be seen from Fig. 1, at a given provirus load, possession of *HLA-B*5401* appeared to increase the risk of developing HAM/TSP.

Discussion

This study demonstrates a protective effect of *Cw*08* against HAM/TSP in HTLV-I-infected individuals, and that class I MHC heterozygosity is associated with a reduction in HTLV-I provirus load. The results of this and our previous study (18) suggest that a strong CTL response is protective in HTLV-I infection. There is conflicting evidence on the role of CTLs in HTLV-I infection (8, 37). Recently we have shown that freshly isolated autologous CD8⁺ T cells rapidly kill Tax-expressing naturally HTLV-I-infected CD4⁺ cells by a perforin-dependent mechanism and, furthermore, that there is a negative correlation between the frequency of Tax-11-19-specific CD8⁺ T cells and the percentage of CD4⁺ T cells in the peripheral blood of HTLV-I-infected patients (20). These results favor the interpretation that CTLs are protective in HTLV-I infection. However, it is possible to reconcile both a protective and a pathogenic effect of the CTL response (38).

The level of *HLA-C* expression on the cell surface is ~10% of the level of expression of *HLA-A* and *-B* (39), and the nonsynonymous nucleotide substitution rate in the peptide binding region of *HLA-C* has also been reported to be lower in *HLA-C* than in *HLA-A* or *-B* (40). However, several naturally occurring *HLA-C*-restricted CTL responses directed against viral Ags, in particular HIV, have been described (41, 42), including an *HLA-C*-restricted immunodominant CTL response (43) and *Cw8*-restricted CTL clones (44). In addition, *HLA-C* disease associations have been

Table IV. *Heterozygosity at all three HLA class I loci was associated with a lower proviral load of HTLV-I in HAM/TSP patients^a*

	HAM/TSP		HCs		
	Proviral load [‡] (n)	2p [§]	Proviral load [‡] (n)	2p [§]	p [¶]
Heterozygous all three loci	489.8 (128)	0.017	28.2 (119)	0.078	0.039
Homozygous at one or more loci	716.1 (96)		50.1 (83)		

^a n, Number of subjects; ‡, median proviral copy number per 10⁴ PBMC; §, p level (2-tailed, uncorrected) (Mann-Whitney U test); ¶, p level (1-tailed, uncorrected) (Mann-Whitney U test).

Table V. *B*5401 is associated with an increased odds of HAM/TSP^a*

	HAM/TSP (n)	HCS (n)	p	OR [†] (95% CI)
B*54 ⁺	65	27	0.0003*	2.51 (1.53–4.12)
B*54 ⁻	168	175		
A*02 ⁺ B5401 ⁺	17	10	0.02 [#]	2.97 (1.27–7.0)
A*02 ⁺ B5401 ⁻	52	91		
A*02 ⁻ B5401 ⁺	48	17	0.03 [#]	2.04 (1.10–3.80)
A*02 ⁻ B5401 ⁻	116	84		
A*02 ⁺ B5401 ⁺	17	10	0.32 [#]	0.60 (0.23–1.57)
A*02 ⁻ B5401 ⁺	48	17		
A*02 ⁺ B5401 ⁻	52	91	0.0001*	0.41 (0.27–0.64)
A*02 ⁻ B5401 ⁻	116	84		

^a B*5401 is associated with susceptibility to HAM/TSP overall, and in both A*02-positive and A*02-negative subjects. The protective effect of A*02 is only seen in the B*5401-negative subjects. n, Number of subjects; *, χ^2 with Yates correction (two-tailed); #, Fisher's exact test (two-tailed); †, using the approximation of Woolf.

found, the most significant being the association of *Cw*06* with psoriasis (45, 46), but there have been few clear demonstrations of HLA-C associations with infectious disease (25, 47). The development of molecular typing methods for HLA-C alleles (30, 48) has recently overcome the difficulties of detecting the serologically blank HLA-C alleles, and it is now clear that HLA-C locus heterozygosity can be as high as HLA-A locus heterozygosity (36). Therefore, it is likely that HLA-C contributes significantly to protection against certain viral infections. Our data show a higher provirus load in association with homozygosity at the HLA-C locus in HAM/TSP patients; this argues for an important role of HLA-C in Ag presentation. Why did we not detect an association between HLA-C and disease protection in stage I of the study? The Fp associated with *HLA-Cw*08* (12.6%) was less than half of that seen in association with *HLA-A*02* (28.2%) (18). This is because *Cw*08* has a lower gene frequency than *A*02* in the Japanese population (35, 36, 49). The reduced odds of disease seen in association with *Cw*08* and *A*02* were of the same magnitude (OR = 0.42 and 0.43, respectively).

Possession of *Cw*08* reduced the risk of HAM/TSP in the Japanese HTLV-I-infected population in a way that is both additive to, and independent of, the protective effect of *HLA-A*02*. In addition, *Cw*08* and *A*02* were each independently associated with a reduc-

Table VI. *HTLV-I provirus load associated with HLA-B*5401 in the presence or absence of HLA-A*02^a*

	HAM/TSP		HCS	
	Proviral load [‡] (n)	p [§]	Proviral load [‡] (n)	p [§]
B54 ⁺	616.6 (61)	0.02	38.0 (27)	0.86
B54 ⁻	524.8 (163)		34.7 (175)	
A*02 ⁺ B5401 ⁺	562.3 (16)	0.87	81.3 (10)	0.13
A*02 ⁺ B5401 ⁻	512.9 (51)		12.9 (91)	
A*02 ⁻ B5401 ⁺	707.9 (45)	0.01	28.8 (17)	0.17
A*02 ⁻ B5401 ⁻	549.5 (112)		53.7 (84)	
A*02 ⁺ B5401 ⁺	562.3 (16)	0.13	81.3 (10)	0.37
A*02 ⁻ B5401 ⁺	709.7 (45)		28.8 (17)	
A*02 ⁺ B5401 ⁻	512.9 (51)	0.67	12.9 (91)	<0.01
A*02 ⁻ B5401 ⁻	549.5 (112)		53.7 (84)	

^a A significantly higher provirus load was observed in all HAM/TSP patients, and in the A*02-negative HAM/TSP patients, in association with B*5401. A lower provirus load was seen in the B*5401-negative HCS in association with A*02, but the presence of B*5401 appeared to abolish the A*02-associated lower provirus load in the B*5401-positive HCS. n, Number of subjects; ‡, median proviral copy number per 10⁴ PBMC; §, p level (2-tailed) (Mann-Whitney U test).

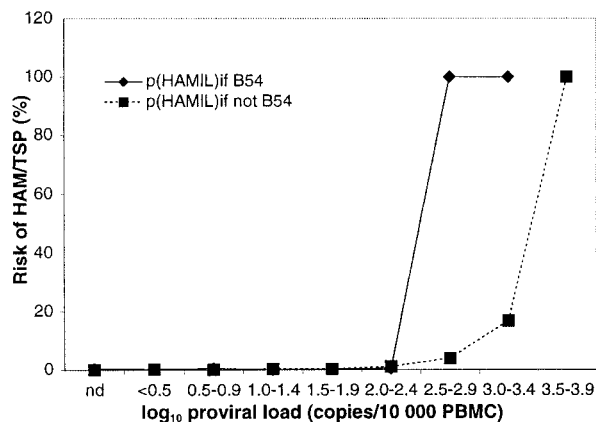


FIGURE 1. The risk of HAM/TSP is increased by *HLA-B*54* at a given proviral load, when the proviral load exceeds a threshold of ~300 copies/10,000 PBMC ($\log_{10} 300 \cong 2.5$). To calculate the risk of HAM/TSP at a given proviral load, Bayes' theorem of conditional probabilities was used as previously described (8, 18). P(HAM|L) denotes the risk of HAM/TSP at a given provirus load. nd, Not detected.

tion in provirus load in the asymptomatic carriers of the virus. The likely explanation for this effect with *A*02* is that HTLV-I-infected lymphocytes are efficiently recognized by A2-restricted CTL and eliminated (18). We propose that the protective effects of *Cw*08* are mediated by a similar mechanism. Experiments are in progress to define the epitopes of the HTLV-I Ags presented by *Cw*08*.

In this study, we tested the hypothesis that class I HLA heterozygosity is beneficial in HTLV-I infection. The class I-restricted T cell response exerts selection pressure on the viral population, which mutates rapidly; successive mutations may lead to eventual escape from effective immune control (50, 51). Therefore, heterozygosity at the class I loci, which allows a broader CTL response to develop (52), may allow a more effective CTL control of viral replication (25) and delay the development of CTL escape mutants (53). The results presented here show that in both asymptomatic carriers (one-tailed level of significance) and HAM/TSP patients, individuals who are fully heterozygous at HLA class I loci had a significantly lower provirus load than individuals homozygous at one or more class I loci. HLA class I heterozygosity was not significantly associated with protection from disease in this study but this may have been due to limitations on sample size. Logistic regression analysis (data not shown) confirmed that individuals who were fully heterozygous at HLA class I loci had a significantly lower provirus load, even after the effects of *HLA-A*02* and *Cw*08* are accounted for. The analysis of heterozygosity in this study was performed at the HLA type level, rather than at the subtype or allele level, because HLA subtypes bind and present to immune effector cells a broadly similar range of epitopes. Also, there is increasing evidence that certain apparently unrelated MHC proteins have very similar specificities in terms of the main anchor residues of their peptide ligands, and it is possible to place up to 70% of HLA-A and -B alleles into one of four HLA supertypes: A2, A3, B7, and B44 (54). Further grouping of alleles in functional categories based on shared peptide binding regions may increase success in searching for disease associations (55).

Many polymorphic loci might influence susceptibility to HAM/TSP. TNF is a strong candidate gene because the expression of this proinflammatory cytokine is induced by HTLV-I Tax protein (56). In this study there was a nonsignificant increase in the frequency of individuals with HAM/TSP carrying the *TNF -857T* allele.

Other groups have observed that apparent *TNF* -857T associations with diabetes mellitus or rheumatoid arthritis are better accounted for by alleles in linkage disequilibrium with *TNF* -857T, namely, *B*5401* and *DRB1*0405* (57, 58).

In this study *B*5401* was significantly associated with susceptibility to HAM/TSP, and none of the other loci in the haplotype described in *Results* was significantly associated with disease. *HLA-B*5401* is found almost exclusively in East Asians (59). Hatta et al. (60) have recently suggested that this *B*54*-associated haplotype is particularly common in the Ryukyuan population, who form one of the important ancestral populations of Okinawa and Kyushu in Southern Japan. Associations have also been described between *B*5401* and other inflammatory conditions in Japan, including hepatitis C (61, 62) and diffuse panbronchiolitis (63, 64). The HLA-associated susceptibility gene associated with diffuse panbronchiolitis has been mapped to a 200-kb region, 300 kb telomeric of the HLA-B locus (65). As well as the described linkage disequilibrium with *TNF* -857T, *B*5401* is also known to be in linkage disequilibrium with the centromeric MHC class I chain-related gene A (*MICA*) allele *MICA*012* in the Japanese population (66). The recent publication of the complete sequence and gene map of the MHC revealed only pseudogenes in the 46-kB region between HLA-B and *MICA* (67). Therefore, it will be necessary to test the hypothesis that the effects associated with *B*5401* in HTLV-I infection are due to *MICA* polymorphisms.

Our data (Table VI) show that *B*5401* was associated with a higher proviral load in HAM/TSP patients and that it abolished the *A*02*-associated reduction in provirus load in HCs (18). However, even after the proviral load was taken into account, *B*5401* appeared to be associated with a significantly increased risk of HAM/TSP (Fig. 1). Thus, *B*5401* appeared to increase the risk of HAM/TSP both through an effect on provirus load and through an additional effect that is independent of provirus load. The susceptibility to HAM/TSP associated with *B*5401* appeared to overcome the protective effect associated with *A*02*, and the reduction in provirus load associated with *A*02* in HCs was only seen in the *B*5401*-negative population. These observations suggest that the *B*5401*-associated susceptibility effect was dominant over the *A*02*-associated protective effect. The reason for the association between *B*5401* and HAM/TSP remains uncertain. Because the *B*5401*-containing haplotype is also associated with a number of other inflammatory conditions (see above), which are not necessarily associated with HTLV-I infection, we suggest that *B*5401* or a closely linked gene contributes to inflammation in an Ag-nonspecific manner.

Although the observed differences in proviral load associated with the presence of individual HLA alleles in this study were statistically significant, and in the direction consistent with their effects on the risk of HAM/TSP, the differences between the median values are sometimes small (Tables II, IV, and VI). However, the median proviral load in HAM/TSP patients was only 16-fold higher than that of HCs (6) and logistic regression analysis (A. L. Lloyd, unpublished data) shows that the 4-fold reduction in proviral load associated with *HLA-Cw*08* is associated with a 2.4-fold reduction in the odds of HAM/TSP, in close agreement with the OR calculation (Table I). The proviral load differences associated with *B*5401* (Table VI) are smaller, therefore the biological significance is less clear. However, because the risk of HAM/TSP rises rapidly when the proviral load exceeds an apparent threshold of ~1 copy per 100 PBMCs (6), a small rise in load might be accompanied by a substantial increase in the risk of HAM/TSP.

In conclusion, we have now found three class I alleles to be independently associated with development of HAM/TSP, two associated with protection (*A*02* and *Cw*08*) and one with susceptibility (*B*5401*), and a susceptibility haplotype (*Cw*0702-B*0702-DRB1**

*0101-DQB1*0501*) within which *DRB1*0101* appears to have the strongest effect (18). Furthermore, we have demonstrated an effect of HLA class-I heterozygosity in lowering provirus load, which is known to be an important factor in the risk of developing disease. These associations may not be replicated in other populations whose HLA frequencies differ from those in this Japanese population; for example, *B*5401* occurs almost exclusively in East Asian populations. However, *A*02* was also significantly associated with a lower prevalence of HAM/TSP in a small population of Afro-Caribbean origin in London (18). Other genetic factors that are important in the immune response to viruses may also be important in influencing the outcome of HTLV-I infection. The pathogenesis of HAM/TSP remains unknown, but these data on the association of HLA class I alleles with disease susceptibility/protection favor the interpretation that a strong CTL response in HTLV-I infection is beneficial.

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